

Appl. No. 09/714,040
Amendment dated February 1, 2006
Reply to Office Action of November 2, 2005

Amendments to the Claims:

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

1-24. (cancelled)

25. (currently amended) A composition comprising monospecific $F(ab')_2$ wherein the $F(ab')_2$ is:

- (a) is free of $F(ab')_2$ having hinge region intrachain disulfide bonds; and
- (b) comprises a first and a second Fab', each first and second ~~$F(ab')$~~ Fab' comprising a CH1 domain fused to an ~~C-terminal~~ amino acid sequence of about 1 to 10 amino acids, wherein the amino acid sequence of 1-10 amino acids comprises comprising a C terminal amino acid sequence Cys-X-X, wherein ~~one or both Xs are absent or~~ X is Ala, Arg, Pro or Asp, and the cysteine of the first Fab' forms a ~~disulfide~~ bond with the cysteine of the second Fab' to form the the monospecific $F(ab')_2$.

26-28. (cancelled)

29. (previously amended) The composition of claim 25, wherein each first and second Fab' comprises the C-terminal amino acid sequence Cys-Ala-Ala.

30-37. (cancelled)

38. (previously presented) The composition of claim 25, wherein the C-terminal amino acid sequence comprises Cys-Pro-Pro.

39. (previously presented) The composition of claim 25, wherein the $F(ab')_2$ polypeptide lacks a heavy and light interchain disulfide bond.

Appl. No. 09/714,040
Amendment dated February 1, 2006
Reply to Office Action of November 2, 2005

40. (currently amended) A composition comprising a $F(ab')_2$ comprising a first and second $F(ab')$ Fab', wherein each first and second Fab' comprises a CH1 region fused to an amino acid sequence consisting of Cys-X-X, wherein one or both Xs are absent or X is Ala, Arg, Asp or Pro.

41. (previously amended) The composition of claim 40, wherein the amino acid sequence consists of Cys-Ala-Ala or Cys-Pro-Pro.

42. (previously presented) The composition of claim 40, wherein the $F(ab')_2$ lacks a heavy and light interchain disulfide bond.

43. (previously presented) The composition of claim 25, wherein the $(Fab')_2$ lacks glycosylation.

44. (new) A composition produced by the process of:

- a) expressing a nucleic acid sequence encoding a Fab' in a microbial host cell under conditions suitable for secretion of the Fab' to the periplasmic space; wherein the Fab' comprises a CH1 domain attached to an amino acid sequence of about 1-10 amino acids, wherein the amino acid sequence of 1-10 amino acids comprises a C terminal amino acid sequence of Cys-X-X, wherein X is Ala, Arg, Asp or Pro;
- b) recovering the Fab' from the host cell and forming a covalent bond between a free thiol of each Fab' to form a monospecific $F(ab')_2$ or forming a covalent bond between a free thiol of the Fab' with a heterologous molecule.

45. (new) The composition of claim 44, wherein the Fab' comprises the C terminal amino acid sequence Cys-Ala-Ala.

46. (new) The composition of claim 44, wherein the Fab' comprises the C terminal amino acid sequence Cys-Pro-Pro.

Appl. No. 09/714,040
Amendment dated February 1, 2006
Reply to Office Action of November 2, 2005

47. (new) The composition of claim 44, wherein the heterologous molecule is a detectable label, cytotoxic drug, toxin, or solid support.

48. (new) The method of claim 44, wherein the heterologous molecule is a radionuclide or fluorescent probe.